USM SHORT TERM:
304/PPSG/6131209

THREE DIMENSIONAL CRANIOFACIAL MORPHOMETRICS: ANALYSIS OF MALAY CLEFT LIP AND PALATE INFANTS

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BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
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Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** Abdul Hakim bin Abdul Basir

Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* :
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2) **Pusat Pengajian/Pusat/Unit :** P.P. Sains Pergigian

3) **Tajuk Projek:** Three Dimensional Craniofacial Morphometrics: Analysis of Malay Cleft Lip and Palate Children.

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

ABSTRACT

Three dimensional craniofacial morphometry was investigated in a sample of 29 cleft lip and palate (CLP) infants aged between 0 – 12 months. They were compared with 12 non-cleft (NC) infants in the same age group. Every one of them underwent CT scan procedure to obtain three dimensional data. This data was then measured in PERSONA software, developed by the Australian Craniofacial Unit, Adelaide. Significant differences in the craniofacial-cervical morphology of infants with CLP compared with NC infants were noted as well as the differences between affected males and females. However these differences need to be further recognized in the future since they can improve our understanding of developmental associations in CLP and also assist in the management of individuals with CLP.

ABSTRAK

Morfometri tiga dimensi bahagian muka dan kepala dijalankan ke atas 29 pesakit rekahan bibir dan lelangit (CLP) berumur diantara 0 – 12 bulan. Morfometri sama dilakukan ke atas 12 kanak-kanak normal (NC) yang sebaya dan dalam julat umur yang sama. Setiap mereka menjalani imbasan CT untuk memperoleh maklumat dan data tiga dimensi. Data itu kemudiannya diukur dalam perisian PERSONA yang dimajukan oleh Australian Craniofacial Unit, Adelaide. Perbezaan signifikan pada morfologi kepala dan leher kanak-kanak CLP dan NC amatlah ketara. Begitu juga, perbezaan dapat dikesan jika perbandingan di antara lelaki dan perempuan CLP dibuat. Bagaimanapun perbezaan ini perlu dikenalpasti dalam kajian-kajian akan datang supaya kefahaman kita dalam faktor-faktor perkembangan dapat dipertingkatkan serta membantu dalam pengurusan kesihatan individu-individu CLP.

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
rekahan bibir dan lelangit	cleft lip and palate
perkembangan kraniofasial	craniofacial growth
–	computed tomography
populasi Melayu	Malay population
pembedahan kraniofasial	craniofacial surgery

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

– Lampiran A –

6. **Peralatan Yang Telah Dibeli:**

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

..... Terima kasih di atas laporan .....
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T/TANGAN PENGERUSI
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UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN PENYELIDIKAN GERAN J/PENDEK
PENYATA PERBELANJAAN SEHINGGA 31 DISEMBER 2004

Jumlah Geran		14,000.00	Ketua Projek	EN HAKIM B ABD .BASIR
Peruntukan 2002 (Tahun 1)	RM	14,000.00	Tajuk Projek	THREE DIMENSIONAL CRANIOFACIAL MORPHOMETRICS:PRE-AND POST OPERATIVE ASSESSMENT OF MALAY CLEFT LIP AND.....
Peruntukan 2003 (Tahun 2)	RM	0.00		
Peruntukan 2004 (Tahun 3)	RM	0.00	Tempoh	FEBRUARI 2002- 31 JULAI 2003
			No.Akaun:	304/PPSG/6131209

Kwgan	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSG	6131209		-	-	-	-	-	-	-
304	14000	PPSG	6131209		-	-	-	-	-	-	-
304	15000	PPSG	6131209		-	-	-	-	-	-	-
304	21000	PPSG	6131209		870.00	3,887.40	(3,017.40)	-	239.80	239.80	(3,257.20)
304	22000	PPSG	6131209		-	-	-	-	-	-	-
304	23000	PPSG	6131209		4,800.00	958.56	3,841.44	-	-	-	3,841.44
304	24000	PPSG	6131209		-	-	-	-	-	-	-
304	25000	PPSG	6131209		-	12.00	(12.00)	-	-	-	(12.00)
304	26000	PPSG	6131209		-	-	-	-	-	-	-
304	27000	PPSG	6131209		3,300.00	4,489.55	(1,189.55)	-	-	-	(1,189.55)
304	28000	PPSG	6131209		-	-	-	-	-	-	-
304	29000	PPSG	6131209		3,900.00	4,359.52	(459.52)	-	-	-	(459.52)
304	35000	PPSG	6131209		1,130.00	-	1,130.00	-	-	-	1,130.00
					14,000.00	13,707.03	292.97	-	239.80	239.80	53.17

PRESENTATIONS WITH ABSTRACTS

- 7th National Conference on Medical Sciences, Kota Bharu, Malaysia, 17-18th May 2002
  - CT Analysis of infants with cleft lip and palate.
  - Comparison of the position of the hyoid bone and hard palate in infants with cleft lip and palate and infants without cleft lip and palate

- IADR (ANZ Division), 42nd Annual Scientific Meeting, Sydney, Australia, 29th Sept - 2nd October, 2002
  - Hyoid bone position in infants with cleft lip and palate

- 16th Annual Scientific Meeting, Australasian Society for Human Biology, Perth, 9-11th December 2002
  - CT analysis of infants with cleft lip and palate

- Australasian Society for Medical Research, Adelaide, Australia, 30th May, 2003
  - A 3D CT analysis of the hyoid bone in children with cleft lip and palate

- Australasian Cleft Lip and Palate Association Conference, Sydney, 8 – 9th August 2003
  - A 3D CT analysis of the nasopharynx in children with cleft lip and palate

- Colgate Australian Clinical Dental Research Centre Research Day, 22 August 2003
  - 3D CT analysis of the cervical spine in children with cleft lip and palate

- International Association for Dental Research, Australian and New Zealand Division, Melbourne, 28th September – 1st October 2003
  - 3D CT analysis of the cervical spine in children with cleft lip and palate

- Australasian Society for Medical Research, Adelaide, Australia, 23rd-25th November, 2003
  - A 3D CT analysis of the nasopharynx in children with cleft lip and palate
  - 3D CT analysis of the cervical spine in children with cleft lip and palate

- 14th Biennial Congress - Asian Surgical Association, Kota Kinabalu, Sabah, Malaysia, 4th – 6th December 2003
    - A 3D CT analysis of the nasopharynx in children with cleft lip and palate
    - A 3D CT analysis of the hyoid bone in children with cleft lip and palate

ADDITIONAL PRESENTATIONS

- The Universiti Sains Malaysia Craniofacial Surgery Course-Team Building, Kota Bharu, Malaysia, 13th-15th July, 2002
    - Application of 3D CT Imaging in the study of craniofacial dysmorphology
- Australian Craniofacial Symposium. Australian Craniofacial Unit, Women's and Children's Hospital, Adelaide, 16 May 2003
    - Overview of CT morphology of cleft lip and palate
- Australia Dental Association, Limestone Coast Seminar, Mount Gambier, 17 – 18 October 2003
    - Progress in understanding craniofacial malformation
- Research Seminar, Flinders Institute for Health and Medical Research, Human Communication Research Group, Flinders Medical Centre, Adelaide, 8th March 2004
    - 3D CT analysis of anatomical structures in patients with cleft lip and palate

PAPERS IN PREPARATION FOR PUBLICATION

Rajion ZA, Netherway DJ, Townsend GC, Shuaib IL, Halim AS, Samsudin AR, McLean NR, David DJ (2004). A 3D computed tomographic analysis of the hyoid bone in patients with cleft lip and palate. *Cleft Palate-Craniofacial J.* (In preparation)

Rajion ZA, Netherway DJ, Townsend GC, Shuaib IL, Anderson PJ, Halim AS, Samsudin AR, David DJ (2004). A 3D computed tomographic analysis of the cervical spine in patients with cleft lip and palate. *Cleft Palate-Craniofacial J.* (In preparation)

Rajion ZA, Netherway DJ, Townsend GC, Shuaib IL, Halim AS, Samsudin AR, McLean NR, David DJ (2004). A 3D computed tomographic analysis of the nasopharynx in patients with cleft lip and palate. *Cleft Palate-Craniofacial J.* (In preparation)

Rajion ZA, Netherway DJ, Townsend GC, Shuaib IL, Halim AS, Samsudin AR, McLean NR, David DJ (2004). A 3D computed tomographic analysis of the cranial base in patients with cleft lip and palate. *Cleft Palate-Craniofacial J.* (In preparation)

Rajion ZA, Netherway DJ, Townsend GC, Shuaib IL, Halim AS, Samsudin AR, McLean NR, David DJ (2004). A 3D computed tomographic analysis of the spheno-occipital synchondrosis in patients with cleft lip and palate. *Cleft Palate-Craniofacial J.* (In preparation)

Three Dimensional Craniofacial Morphometrics: Analysis of Malay Cleft Lip and Palate Infants

1.0 Introduction

Cleft lip and palate (CLP) represents one of the most common forms of facial deformity affecting one in every 500 to 1000 live births worldwide. It affects individuals in all societies and has been the subject of considerable research. The focus of previous studies has been on the aetiology, investigating the implications and consequences for affected individuals, and surgical management.

The results of embryological studies have provided a clearer picture of what happens during craniofacial development. This was highlighted by Diewert (1983) who reported changes in craniofacial dimensions, proportions, and spatial relations during the development of the secondary palate. Movements of the palatal shelves to the horizontal position involve a complex interaction between the shelves and the tongue that is influenced by developmental events in the shelves and the surrounding craniofacial complex. Normal facial growth tends progressively to separate the palatomaxillary processes from the tongue-mandibular complex as the naso-maxillary complex lifts upward and the tongue shifts forward prior to shelf elevation. This positional change may enhance palatal shelf elevation.

In addition to studies in humans, investigations using animal models show that, during the period of shelf elevation, there is almost no growth in head width, but constant growth in head height. This means that the position of least resistance for the expanding palatal shelves is to occupy the space above the tongue (Ferguson, 1988).

Our understanding of the cellular and molecular events involved in craniofacial development has improved greatly because of rapid advances in molecular biology. During recent years, enormous progress has been made in our understanding of normal and abnormal development of the head and neck. This progress has been made possible through technical developments, particularly the application of molecular techniques, and the development of animal models for studying the roles of genetic and environmental factors relevant to human CLP formation. The application of precise cell marking procedures has led to a much better appreciation of the cell movements and interactions involved in germ layer formation. The techniques of scanning electron microscopy and *in situ* hybridisation methods for studying gene expression have demonstrated the extensive contributions of neural crest cells to craniofacial development.

In CLP studies, anatomical differences have been observed. Excessive separation of structures formed lateral to the tongue was observed by Maue-Dickson and Dickson (1980) in a 15-week-old human foetus with cleft palate. Subtelny (1955) also found that the nasopharynx was abnormally wide and the width between the maxillary tuberosities was increased in unoperated CLP subjects.

Malformation resulting in CLP results from perturbations or insults during embryonic development between the fourth and tenth weeks of gestation. Cleft lip and cleft of the primary palate results from a failure of fusion of medial nasal, lateral nasal and maxillary processes on either left, right or both sides of the forming craniofacial complex. After primary palate fusion, secondary palate fusion takes place during the ninth week to tenth week of gestation. Cleft palate may result from disturbances at any stage of palate development: defective palatal shelf growth, delayed or failed shelf elevation, defective shelf fusion, failure of medial edge cell death, post-fusion rupture and failure of mesenchymal consolidation and differentiation (Ferguson, 1988).

CLP can occur in syndromic and non-syndromic forms. This study concentrated on non-syndromic forms as they are less likely to have other pathological problems that can affect the results. However, there may be some common mechanisms in both types. Non-syndromic clefts of the oral cavity seem to be aetiologically distinctive; however, clinically they make up the majority of cleft cases in the human population. The non-syndromic forms of CLP have a multifactorial mode of inheritance with both genetic and environmental factors operating. Currently, genes implicated in CLP have been identified on different chromosomes, including chromosomes 6 and 11 (Juriloff and Mah, 1995; Eiberg *et al.*, 1987; Chenevix-Trench *et al.*, 1992). Genetic analyses of non-syndromic oral clefts have produced significant results such as association studies that point to polymorphisms at the TGF alpha locus playing a key role in the aetiology of oral clefts. There is a suggestion that this locus may interact with exposure to maternal smoking to influence the risk oral clefting (Shaw *et al.*, 1996). The lack of consistent results from family studies highlights the fact that non-syndromic CLP is a heterogenous condition, undoubtedly caused by more than one factor.

Many affected individuals appear as spontaneous events with no affected family members. Multiple 'chance' combinations of genetic and environmental factors (multifactorial aetiology) appear to be responsible for most of these CLP cases. The

most implicated environmental factors for human CLP have been cigarette smoking, alcohol and nutritional factors such as folate deficiency (Wyszynski *et al.*, 1996).

This aetiology suggests that it is unlikely that the phenotypic effects will be limited only to the cleft. It is likely that other structures will also be affected. It is also likely that there will be a range of expressions of CLP, in other words phenotypic heterogeneity.

The overall phenotypic pattern in CLP has not been well understood. The structures affected in the cranio-cervical region have not been well described previously. The present study reports several anatomical anomalies not previously recognised. It is not known whether these changes are a result of the CLP, a cause, or simply pleiotropic effects associated with the clefting.

It has only been relatively recently that imaging techniques and 3D analytic techniques have enabled a detailed assessment of the skeletal structures in CLP patients. Most early knowledge has come from analyses of conventional radiographs eg lateral head and AP views, which have several limitations such as superimposition of structures, difficulty identifying landmarks and poor visualization of 3D structures.

The availability of 3D methods allows better opportunities to evaluate craniofacial structures. There is now an opportunity of exploring the phenotypes of CLP individuals in much more detail and to describe links, in terms of understanding the mechanisms involved between what is happening at a molecular level and what happens at the phenotypic level. There is a much better opportunity to link the genotype, the molecular mechanisms and the phenotype.

By using 3D CT approaches, variables can now be defined that describe the size and shape of bones and regions. Statistical analyses enable comparisons to be made and help to clarify associations between structures. Multivariate analyses and morphometric analyses are now possible with sophisticated computer software.

The particular advantage offered by this study is that CT data were obtained from CLP individuals at infancy before they had been operated, and records were available for unoperated non-cleft (NC) children, matched for age, for comparison. This study also used a sophisticated software package that enabled accurate and reproducible location of landmarks from which variables could be derived thereby offering advantages over conventional radiographs. This has allowed views that are

not possible with a conventional approach, including images of the hyoid bone, cervical spine, nasopharynx, cranial base and spheno-occipital synchondrosis (SOS).

The description of the associations between the hyoid bone, cervical spine, nasopharynx, cranial base, spheno-occipital synchondrosis (SOS) and CLP, which have not been detailed in previous studies, is possibly the most important contribution of this thesis. These areas were also selected because of their clinical importance to swallowing, hearing, and speech in CLP. This study focussed on the areas more distant from the cleft but within the craniofacial/cervical region. The selection was also based on the hypothesis that CLP reflects part of a broader problem, not just one in the region of the cleft. Previous studies have indicated that CLP is associated with a variety of other anomalies (Maue-Dickson, 1979; Maue-Dickson and Dickson, 1980; Horowitz *et al.,* 1976; Krogman *et al.,* 1975; Molsted *et al.,* 1993, 1995).

2.0 Methodology

2.1 Ethical Approval

Ethical approval was given by the Ethics and Research Committee USM dated 30/8/01, Number: USM/PPSG/Ethics Com/2001[61.3(1)] (Appendix I). Data collection took place in Malaysia from September 2001 to August 2002.

2.2 Data Collection

CT scans were obtained from 29 patients with unoperated non-syndromic cleft lip and palate. Any syndromic patients were excluded. They were aged between 0-12 months and compared with 12 non-cleft patients (NC) in the same age group. The NC patients had normal craniofacial morphology but had medical indications for scanning including meningitis and hydrocephalus.

The distribution of clefts was cleft lip and/or alveolus (CL), n=7; unilateral cleft lip and palate (UCLP), n=10; bilateral cleft lip and palate (BCLP), n=4; isolated cleft palate (ICP), n=8; non-cleft patients (NC), n=12. Cephalometric analyses of cleft lip and/or alveolus (cleft of the primary palate) have been shown to be different in craniofacial morphology from other cleft types (Dahl, 1970; Smahel et al., 1991), for that reason the CL was included in this study. Age and sex distribution of the cleft and NC groups are shown in Table 2.1.

Table 2.1 Age and sex distribution of the cleft and NC groups

Group	Sex	No.	Mean Age (Days)	No.	Range (min-max)
CLP	F(12) M (17)	29	115	76	14-340
NC	F(3) M (9)	12	145	86	19-297

Table 2.1 shows that the age range was greater in the CLP group and a few older children were included. The reason for this is that in CLP group the primary operation had been postponed because of other health problems such as upper respiratory tract infection and aspiration pneumonia.

2.3 Imaging Procedure

Axial scans were obtained with a GE Lightspeed Plus CT Scanner System at the Department of Radiology, Hospital USM. The protocol (Appendix II) used at the Australian Craniofacial Unit (ACFU), Women's and Children's Hospital, Adelaide (Australia), was followed as the basis for the scanning procedure.

2.4 Image Measurement

The PERSONA software package developed by the Research Unit at the ACFU, Women's and Children's Hospital, Adelaide (Abbott et al, 1990, 2000; Netherway et al., 1997, 1999) was utilized for three-dimensional reconstruction of the images and to determine the 3D coordinates of osseous landmarks on a Silicon Graphics Computer workstation.

2.5 Statistical analysis

A linear model (PROC GLM, SAS 2001) incorporating the fixed effects 'sex' and 'cleft group', and using 'age' as a covariate, was fitted to all variables.

The model was as follows:

Variable = Age (14-340 days)
Sex (male, female)
Group (NC, UCLP, BCLP, ICP, CL)

Higher order interactions were not analysed for this small data set. Linear contrast were arranged to compare the control group (NC) with all other groups, and to compare the ICP group (a morphology distinct cleft-type) with other cleft groups. A Chi-square test was used to test for any associations between anomalies of the cervical spine and the incidence of cleft lip and palate. The level of significance was set at 5%.

2.6 Errors of the method

Two determinations were performed to assess the reproducibility of landmark determination and variables derived from these landmarks using Dahlberg's method of double determination (1940). All measurements were repeated after a period of one month. Student's t-tests were used to detect systematic errors (i.e. to ascertain whether the mean difference between repeated measures deviated significantly from zero).

3.0 Results

3.1 Hyoid bone

Table 3.1 Adjusted means and standard errors of the hyoid bone variables.

Variables	Groups									
Hyoid	NC (n=12)		UCLP (n=10)		BCLP (n=4)		CL (n=7)		ICP (n=8)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Lwr length lt GH	8.5	0.62	8.8	0.62	10.2	0.96	7.6	0.71	7.0+	0.67
Lwr length rt GH	8.5	0.56	10.0	0.58	8.3	0.90	7.4	0.67	7.7	0.63
Upr length lt GH	8.3	0.62	9.0	0.61	9.9	0.95	7.3	0.70	6.8+	0.66
Upr length rt GH	8.3	0.56	9.7	0.58	8.4	0.91	7.4	0.67	7.2	0.63
Height lt GH	2.8	0.16	2.5	0.15	2.4	0.24	2.7	0.18	2.3	0.17
Height rt GH	2.7	0.16	2.5	0.16	2.2	0.25	2.7	0.18	2.5	0.18
HB height lt	2.4	0.23	2.2	0.28	2.3	0.45	2.4	0.32	2.4	0.30
HB height rt	2.2	0.21	2.1	0.25	2.6	0.40	2.3	0.28	2.4	0.27
HB upr length lt	4.4	0.27	3.9	0.32	4.3	0.52	5.0	0.36	4.1	0.35
HB upr length rt	4.4	0.24	3.6	0.29	3.8	0.46	4.7	0.32	4.2	0.31
HB lwr length lt	4.1	0.29	3.5	0.35	4.0	0.56	4.2	0.40	4.0	0.38
HB lwr length rt	4.1	0.29	3.5	0.34	3.5	0.55	4.0	0.39	3.4	0.37
Hyoid - upr cervical	22.1	0.63	20.8	0.72	21.1	1.16	21.3	0.81	19.2	0.77
Hyoid - lwr cervical	22.5	0.64	21.9	0.73	21.6	1.18	22.1	0.83	20.0	0.79
Hyoid - basion*	27.5	0.72	32.3	0.86	32.2	1.38	29.1	0.97	29.9	0.92
Hyoid - inf SOS	30.7	0.86	32.3	1.04	33.9	1.68	30.4	1.18	31.9	1.12
Hyoid angle (deg) *	92.9	1.76	85.8	2.10	86.2	3.37	88.7	2.37	87.1	2.46

GH= Greater Horn, HB= Hyoid Body, lwr= lower, upr= upper, lt = left, rt= right
*Significant difference at $p<0.05$ between all cleft groups and non-cleft
+ Significant difference at $p<0.05$ between ICP and other cleft groups combined

Table 3.1 shows adjusted means and their standard errors for the four cleft groups and NC group. Using Generalized Linear Modeling analysis (PROC SAS, 2001), no significant difference in the overall left and right length of the greater horn was found between CLP and NC groups (Table 3.1). However, the lower and upper length of the left greater horn was significantly smaller in the ICP group compared to other affected groups ($p<0.05$) (Figs. 3.1 - 3.4).

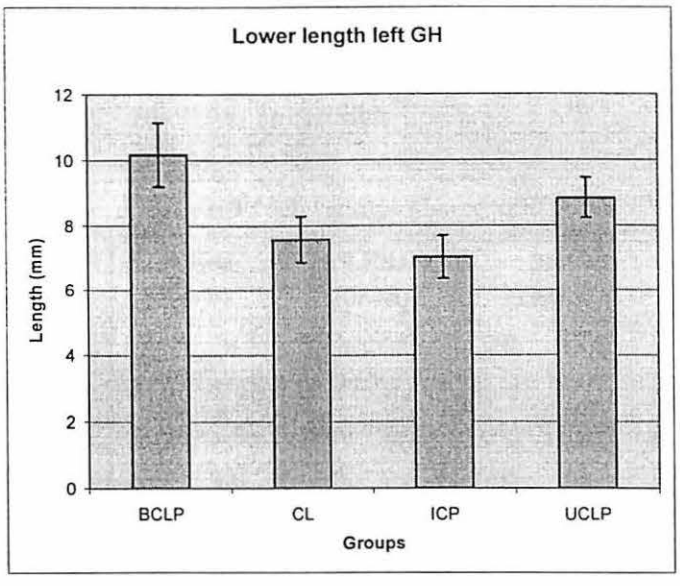

Figure 3.1 Adjusted means and standard errors for the lower length of the left greater horn of the hyoid bone. The ICP group was significantly smaller than the other cleft groups.

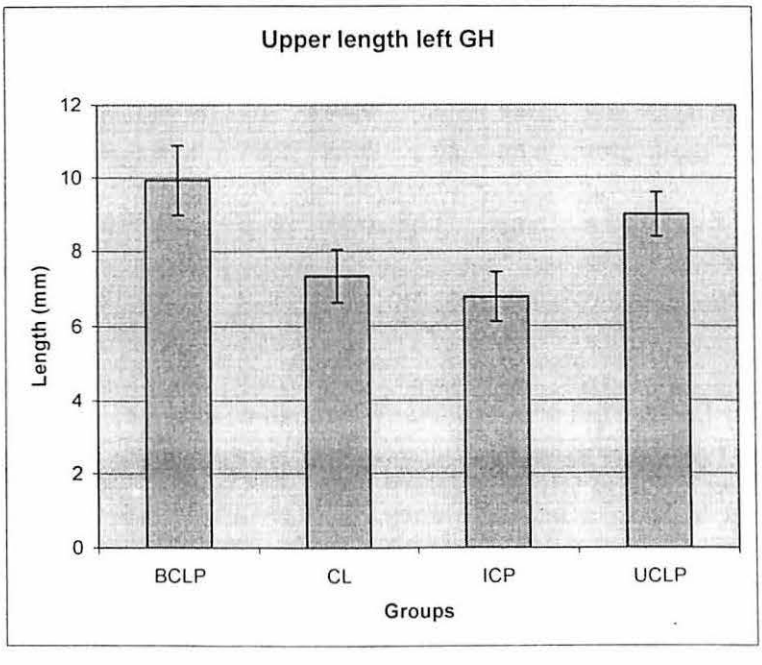

Figure 3.2 Adjusted means and standard errors for the upper length of the left greater horn of the hyoid bone. The ICP group was significantly smaller than the other cleft groups.

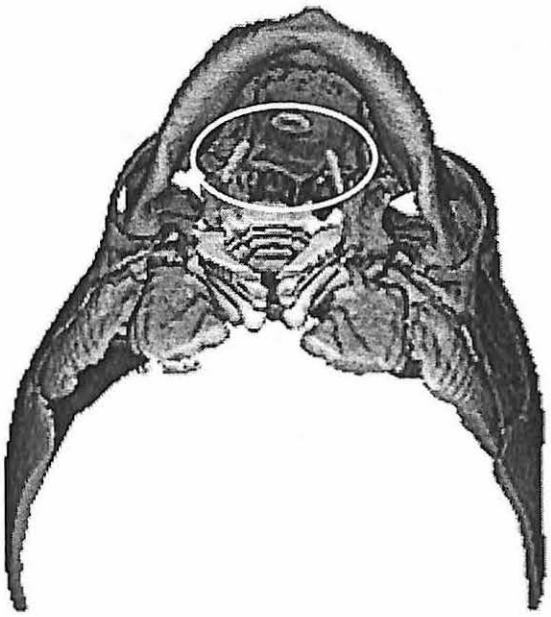

Figure 3.3 Illustrating the normal shape of the hyoid bone in NC patients.

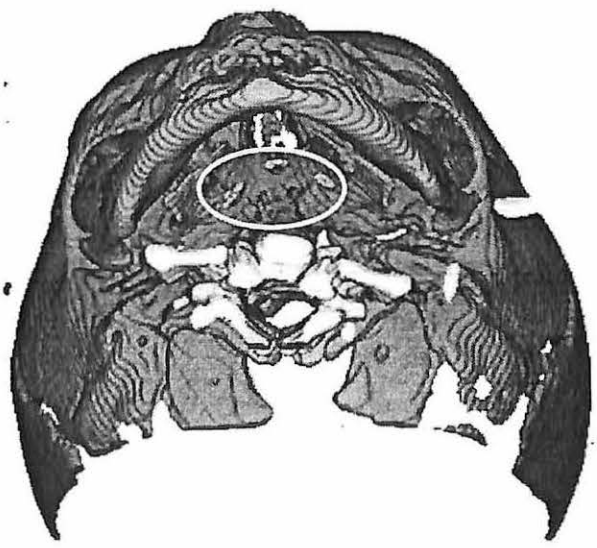

Figure 3.4 Illustrating the smaller size of the hyoid bone in patients with ICP.

3.2 Cervical Spine

Table 3.2 Adjusted means and standard errors of the cervical spine variables.

Variables	Groups									
Cervical Spine	NC (n=12)		UCLP (n=10)		BCLP (n=4)		CL (n=7)		ICP (n=8)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Height C2	13.0	0.40	13.0	0.43	13.1	0.68	13.1	0.50	13.5	0.48
IVS C2/C3	3.1	0.23	3.3	0.25	3.1	0.39	3.2	0.29	2.9	0.27
Height C3*	4.4	0.16	3.7	0.17	3.1	0.27	4.2	0.20	4.0	0.19
IVS C3/C4	2.5	0.18	3.0	0.19	2.9	0.30	2.7	0.22	2.7	0.21
Height C4*	4.5	0.19	3.8	0.19	3.8	0.30	4.3	0.22	4.0	0.21
IVS C4/C5*	2.3	0.16	3.3	0.16	3.1	0.25	2.6	0.19	2.6	0.18
Height C5	4.6	0.16	3.9	0.16	4.0	0.25	4.5	0.19	4.5	0.18
IVS C5/C6*	2.5	0.18	3.2	0.19	3.2	0.30	2.9	0.24	2.5+	0.21
Height C6	4.7	0.20	4.3	0.21	4.2	0.48	4.6	0.26	4.6	0.23
IVS C6/C7	2.9	0.18	3.2	0.17	3.1	0.35	3.1	0.19	2.8	0.17
Height C7*	5.3	0.26	4.6	0.26	3.8	0.54	4.8	0.25	4.5	0.20
Length C2-C6 inf	37.4	0.93	39.6	0.98	37.8	2.18	39.5	1.21	39.3	1.06
Length C2-C7-sup	39.4	1.18	42.4	1.11	40.6	2.34	42.2	1.28	41.5	1.13
Length C2-C7-inf	41.4	1.50	45.0	1.50	38.6	3.05	44.5	1.44	45.0	1.14
Cranio-cervical angle (deg)*	119.0	1.86	111.8	2.00	111.9	3.12	114.6	2.30	112.2	2.35

*Significant difference at $p<0.05$ between all cleft groups and non-cleft

IVS = Intervertebral spaces

+ Significant difference at $p<0.05$ between ICP and other cleft affected groups

Table 3.2 shows adjusted means and standard errors derived from the linear modeling analysis for the four cleft groups and NC group. None of the study variables significant differences between males and females in either the CLP and NC groups and so data are presented for both sexes combined. Using Generalized Linear Modeling analysis (PROC SAS, 2001), the vertebral body heights of C3, C4, C7 in CLP infants were found to be significantly smaller than in the NC ($p<0.05$) (Figs. 3.5 to 3.7).

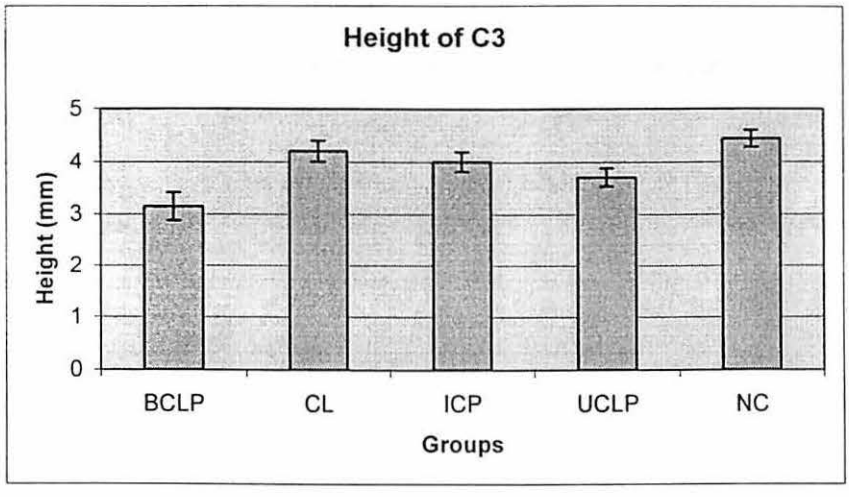

Figure 3.5 The height of vertebral body of C3 was significantly smaller in CLP compared to NC ($p<0.05$).

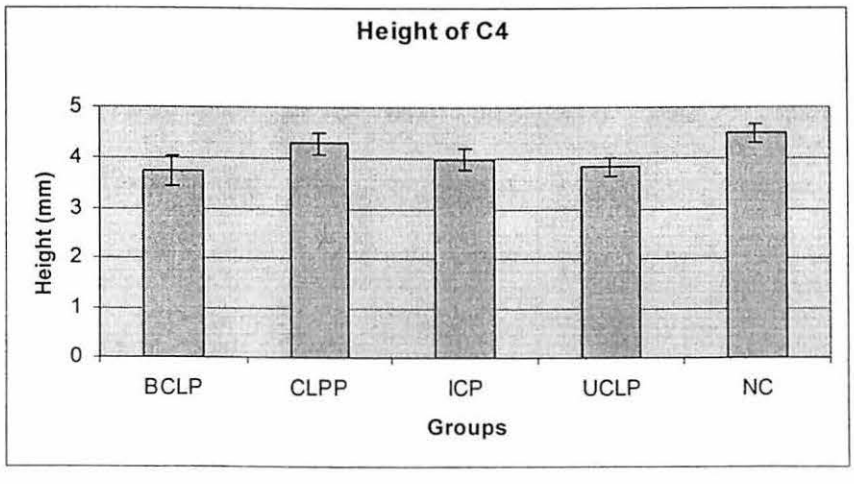

Figure 3.6 The height of vertebral body of C4 was significantly smaller in CLP compared to NC.

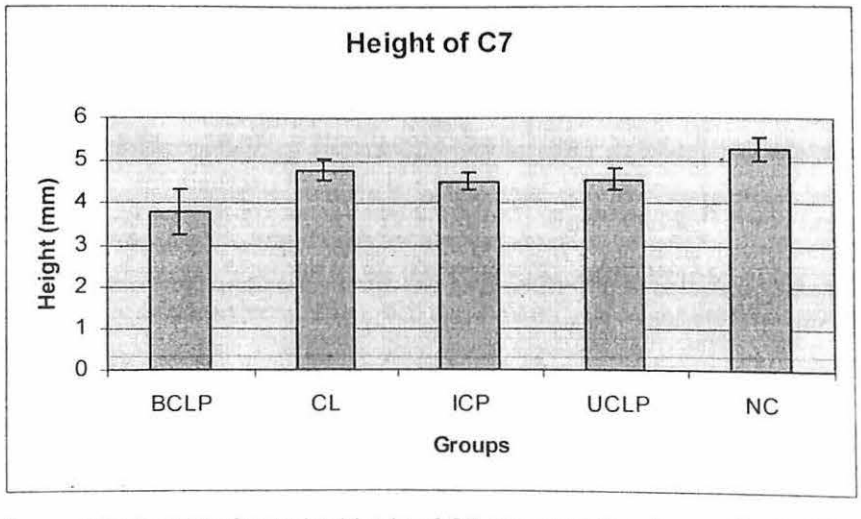

Figure 5.5 The height of vertebral body of C7 was significantly smaller in CLP compared to NC.

3.3 Nasopharynx

Table 3.3 Adjusted means and standard errors of the nasopharyngeal variables.

Variables	Groups									
Nasopharynx	**NC (n=12)**		**UCLP (n=10)**		**BCLP (n=4)**		**CL (n=7)**		**ICP (n=8)**	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Inter hamular notch*	25.6	0.77	33.5	0.83	34.3	1.30	29.6	0.96	29.3+	0.91
Inter hamulus*	22.3	0.59	30.2	0.63	29.8	0.99	25.7	0.73	25.9+	0.70
Inter-lateral pterygoid*	36.0	0.86	43.1	0.92	41.7	1.43	39.9	1.06	39.5	1.00
Hamulus - lateral Ptry.plate lt*	8.3	0.50	7.2	0.53	6.5	0.84	7.4	0.62	7.0	0.59
Hamulus - lateral Ptery.plate rt	8.0	0.49	7.4	0.52	7.3	0.81	7.9	0.60	7.6	0.57
Inter-maxillary tuberosity*	26.4	0.77	35.0	0.83	34.9	1.30	29.9	0.96	30.2+	0.91
Inter-zygomatic distance*	62.3	1.23	70.0	1.32	68.5	2.07	67.4	1.53	66.2	1.44
Vomer - hamulus lt*	18.2	0.47	20.1	0.50	21.5	0.78	20.1	0.58	18.7+	0.55
Vomer - hamulus rt*	17.8	0.47	20.2	0.50	20.6	0.79	19.3	0.59	18.0+	0.55
Vomer - basion	23.0	0.64	24.0	0.69	23.7	1.07	22.9	0.80	26.2+	0.75
Basion - hamulus-lt	26.8	0.65	28.6	0.69	27.7	1.08	28.0	0.80	27.2	0.76
Basion - hamulus rt	26.5	0.63	28.4	0.66	27.9	1.04	27.6	0.77	27.1	0.73
Hamulus angle lt	40.2	1.84	36.0	1.97	37.2	3.08	39.2	2.28	42.7	2.16
Hamulus angle rt	40.8	1.77	38.2	1.90	42.1	2.97	36.1	2.20	45.0+	2.08
Sphenopalatine angle	32.7	1.16	31.0	1.24	27.9	1.94	31.1	1.44	31.5	1.46
Vomerine angle	21.2	1.19	19.4	1.28	17.2	2.00	17.0	1.48	21.4	1.51

* Significant difference at $p<0.05$ between all cleft groups and non-cleft
\+ Significant difference at $p<0.05$ between ICP and combined cleft groups

The widths at the hamular notches (Fig. 3.8), hamuli (Fig. 3.9) and lateral pterygoid plates of the nasopharynx were significantly greater in the CLP groups compared with the NC group ($p<0.05$).

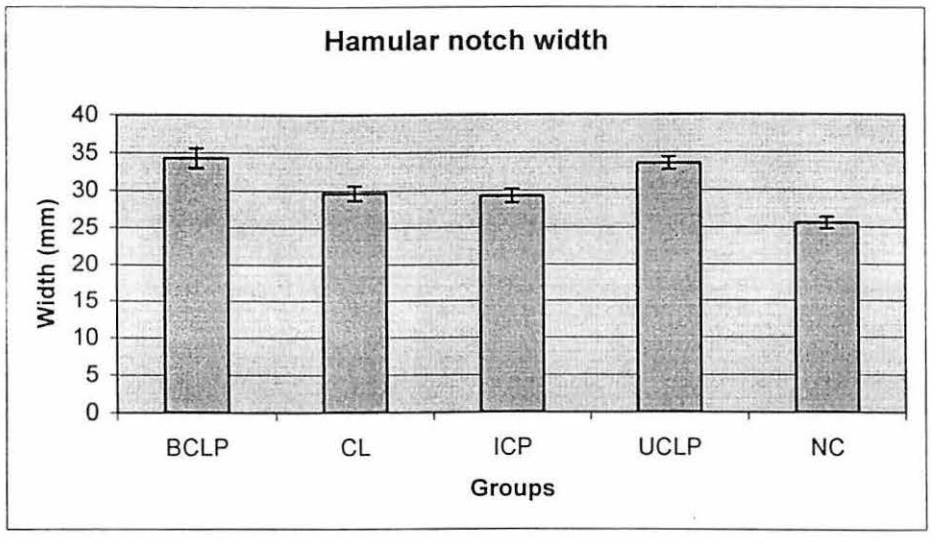

Figure 3.8 Adjusted mean values and standard errors for the hamular notch width in CLP and NC groups. The CLP groups were significantly wider than the NC group and the ICP group was significantly smaller when compared to other CLP groups.

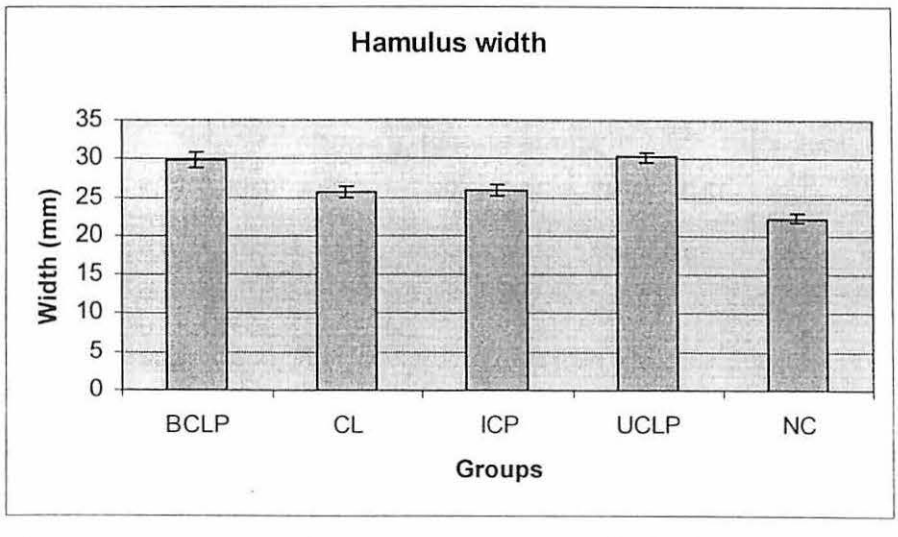

Figure 3.9 Adjusted mean values and standard errors for the hamulus width in CLP and NC groups. The CLP groups were significantly wider than the NC group and the ICP group was significantly smaller when compared to other CLP groups.

3.4 Cranial base

Table 3.4 Adjusted means and standard errors of the cranial base variables.

Variables	Groups									
Cranial Base	**NC (n=12)**		**UCLP (n=10)**		**BCLP (n=4)**		**CL (n=7)**		**ICP (n=8)**	
	$\bar{x}$	**SE**	$\bar{x}$	**SE**	$\bar{x}$	**SE**	$\bar{x}$	**SE**	$\bar{x}$	**SE**
Lt sphenoid height*	8.1	0.29	6.8	0.29	6.9	0.46	6.8	0.33	5.9[+]	0.34
Rt sphenoid height *	7.9	0.26	6.7	0.27	6.8	0.42	7.0	0.31	5.7[+]	0.32
Lt basioccipital height*	7.9	0.27	7.3	0.27	6.7	0.42	7.4	0.31	6.7	0.32
Rt basioccipital height*	7.8	0.25	7.1	0.24	6.7	0.40	7.4	0.29	6.8	0.29
Basion - nasion	64.1	1.31	62.2	1.41	61.8	2.19	62.0	1.63	60.5	1.66
Basion - sella	25.9	0.59	25.8	0.64	24.6	0.99	26.4	0.74	25.8	0.75
Sella - nasion*	44.9	0.92	42.0	0.98	42.5	1.53	41.3	1.14	40.7	1.16
Sella – sup. sphenoid lt	9.2	0.34	9.9	0.34	9.5	0.54	10.2	0.40	9.5	0.41
Sella to sup. sphenoid rt	9.2	0.36	10.0	0.36	9.5	0.57	10.3	0.42	9.5	0.43
Basion – sup. basioccipital lt	15.5	0.43	15.5	0.44	14.9	0.68	15.8	0.50	15.6	0.51
Basion - superior basioccipital rt	15.7	0.47	15.3	0.47	14.4	0.73	15.8	0.54	15.7	0.55
Basion – inf. basioccipital lt	13.3	0.42	14.0	0.42	13.5	0.66	13.8	0.49	14.3	0.50
Basion – inf. basioccipital rt	13.7	0.42	14.0	0.43	13.1	0.67	13.8	0.50	14.1	0.50
Cranial base angle	131.4	2.00	131.9	2.13	134.1	3.34	132.1	2.48	130.7	2.51

*Significant difference at $p<0.05$ between all cleft groups and non-cleft

[+] Significant difference at $p<0.05$ between ICP and other combined cleft groups

When the GLM model was applied to the height data for the basi-sphenoid and basi-occipital bones, statistically significant differences were found between the CLP and NC groups (Table 3.4). The heights of the bones on both sides in CLP infants were significantly smaller when compared to the NC ($p<0.05$). Furthermore, the heights of the basi-sphenoid in the ICP group on both sides were significantly smaller when compared with the other cleft groups ($p<0.05$) (Figs. 3.10 and 3.11).

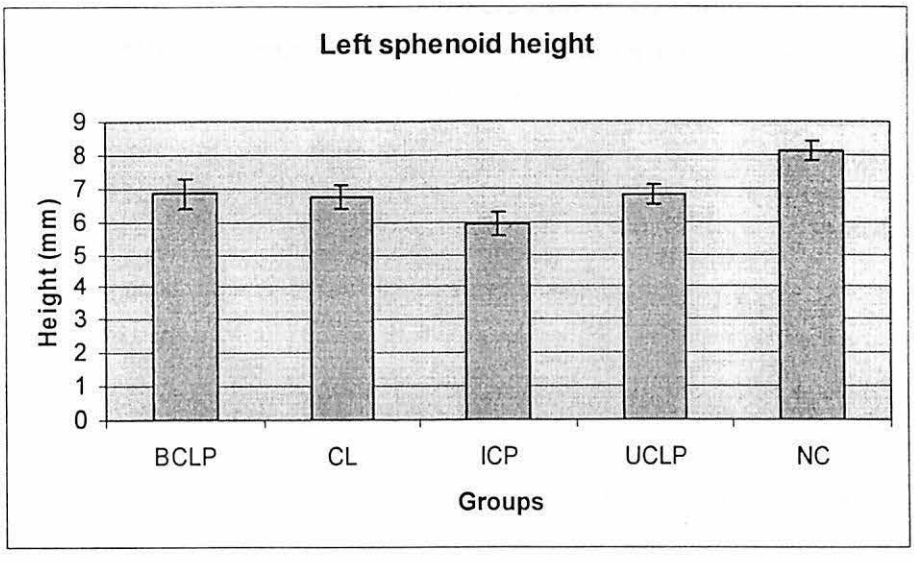

Figure 3.10 Adjusted mean values and standard errors for left sphenoid height. The CLP groups were significantly smaller than the NC group. The ICP group was significantly smaller when compared to the other cleft groups.

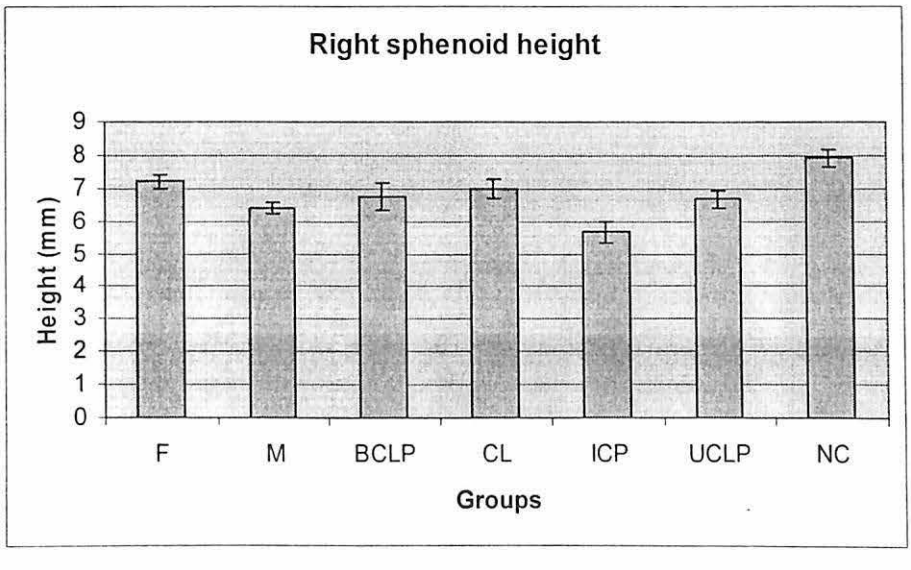

Figure 3.11 Adjusted mean values and standard errors for the height of the right sphenoid bone. The CLP groups were significantly smaller than the NC group. The ICP group was significantly smaller when compared to the other affected cleft groups. The height in females (F) was significantly larger than in males (M).

3.5 Spheno-occipital synchondrosis (SOS)

Table 3.5 Adjusted means and standard errors of the spheno-occipital synchondrosis variables.

Variables	Groups									
SOS	NC (n=12)		UCLP (n=10)		BCLP (n=4)		ICP (n=8)		CL (n=7)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Inferior SOS width*	1.2	0.15	1.3	0.15	1.6	0.23	1.5	0.16	1.7	0.17
Superior SOS width	1.2	0.15	1.2	0.15	1.7	0.24	1.4	0.17	1.6	0.17

* Significant difference at $p<0.05$ between all cleft groups and non-cleft

When the GLM model was applied to the width of the spheno-occipital synchondrosis, statistically significant differences were found between the CLP and NC groups. Greater width was found in the inferior part of the spheno-occipital synchondrosis in CLP infants than in the NC group ($p<0.05$) (Fig 3.12). The width of the inferior SOS in females was narrower than in males ($p=0.09$). The width of the superior SOS was not significantly different between CLP and NC groups ($p=0.09$) (Fig. 3.13). However, there was a significant difference between males and females in the width of superior SOS ($p<0.05$). The SOS in females was narrower than in males.

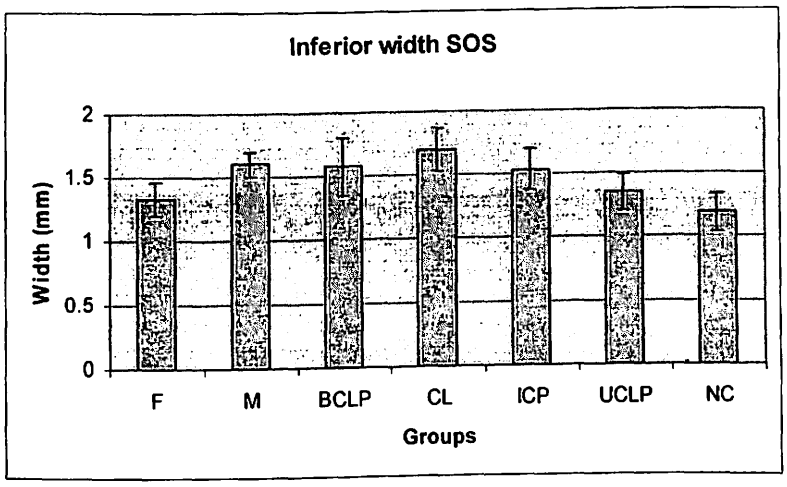

Figure 3.12 Adjusted mean values and standard errors showing the width of the inferior SOS. The CLP group was significantly wider than the NC group. The ICP group was not significantly different to the other cleft groups. There were no significant differences between males and females.

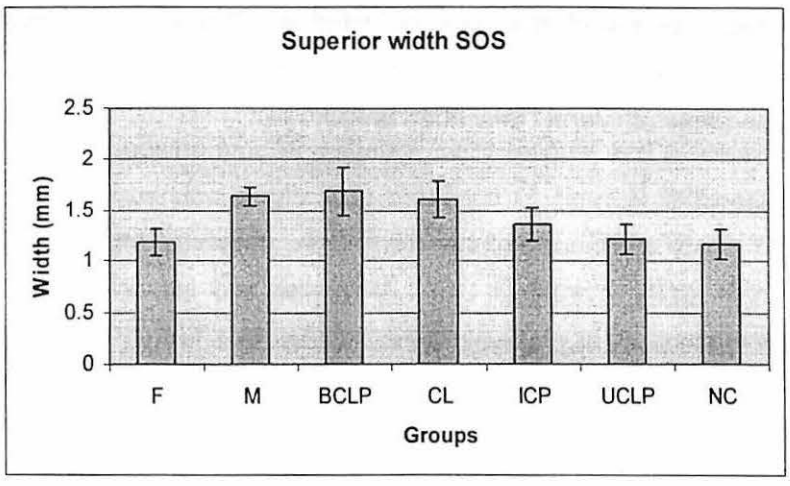

Figure 3.13 Adjusted mean values and standard errors showing the width of superior synchondrosis. The CLP groups were not significantly different to the NC group. The ICP group was not significantly different to the other affected cleft groups. The width in males (M) was significantly larger than in females (F).

4.0 Discussion

After detailed analysis of the data collected for this study, several differences between the CLP and NC groups became apparent. These differences pertained to the five main areas of interest described below:

4.1 Hyoid Bone

This 3D CT study has shown, for the first time, details of the abnormalities of the hyoid bone in CLP. The hyoid bone is smaller and in some cases there is no ossification of the body of the hyoid bone. The hyoid is further from the cranial base. There is smaller angulation and also it is at a low level in relation to the cervical vertebrae.

These phenotypic changes in the hyoid bone relate to structures derived from the first, second and third branchial arches. The hyoid bone is a composite endochondral bone that develops from cartilage of the 2nd and 3rd branchial arches – lesser horn from the 2nd branchial arches; greater horn from the 3rd branchial arches; body from both 2nd and 3rd branchial arches (Koebke, 1978). In terms of embryology, this finding indicates that the underlying factors associated with clefting anomalies not only affect the labiomaxillary and palatine structures of the first arch, but also appear to influence the development of structures derived from the 2nd and 3rd branchial arches.

Clinically there is an association between the low level of the epiglottis and the level of the hyoid in relation to the cervical vertebrae with aspiration pneumonia. Alteration in the position of the hyoid also presents significant potential problems in terms of breathing, swallowing and head posture, because of alterations in attachments of the muscles responsible for these functions.

In terms of clinical problems presented by the CLP groups 4/29 had aspiration pneumonia and 6/29 had upper respiratory tract infections causing surgical intervention to be deferred. When two or more anomalies present together, medical complications can result and their coincidence carries implications for morbidity and prognosis (Azmi *et al.*, 1983). Pandya and Boorman (2001) found failure to thrive (FTT) in babies with CLP, but with a feeding support nurse and airway management it improved. It may also be that neonatal nurses may be able to provide more effective care by understanding more of the nature of CLP and its effects on feeding. The multidisciplinary nature of effective care of CLP infants also involves speech pathology. A greater understanding of the differences in the morphology of the hyoid bone may improve the approaches to speech therapy in CLP infants. Therapy based

on current knowledge entirely overlooks the hyoid malformation. It is hoped that the findings of this study may lead to new approaches to CLP speech therapy.

4.2 Cervical Spine

The cervical spine showed smaller vertebral body heights and greater intervertebral spaces and smaller cervical angle. The presence of cervical spine anomalies was noted and delayed ossification of the anterior arch of C1. There was also an association between the occurrence of CLP and the presence cervical spine anomalies.

Endochondral ossification of the upper cervical vertebrae commences by the eight week of foetal life and is completed by about three to six years of post-natal life (Farman and Escobar, 1982; Sandham, 1986). Although no significant difference was found in the overall length of the cervical spine, the smaller vertebral bodies and greater intervertebral spaces suggest that there may be a difference in the pattern of skeletal ossification or that maturation is delayed or altered in CLP compared with the NC infants. This delay in maturation may influence the lifting of the head (during 6^{th} – 10^{th} weeks *in utero*) and could also possibly be associated with the failure of the elevation of the palatal shelves to meet leading to clefting of the palate. These limitations of the extension of the head of the foetus could also interfere with the descent of the glosso-mandibular complex. The wedging position of the tongue in between the palatal shelves has been shown to be a major factor contributing to failure of shelf elevation and clefting of the secondary palate (Diewert, 1983).

Abnormalities of the cervical spine in CLP, such as fusion of the posterior upper arch and short posterior arch of C1, lipping of the atlas (C1) and anterior arch anomalies of C1 which included two anterior arches instead of one and an asymmetric anterior arch to the right, have not been demonstrated before the use of 3D CT.

The reduced cervical angle in CLP may be associated with postural changes to facilitate airway maintenance. Anderson (1997), in his study on craniosynostosis patients, reported that cervical spine fusion, particularly those affecting the higher levels, may also have important consequences for head posture with resulting influences on craniofacial growth and dental occlusion. Other researchers have also proposed that cervical spine anomalies may alter head posture (Solow *et al.*, 1984; Solow and Siersbaek-Nielsen, 1986; Hellsing *et al.*, 1987; Solow and Siersbaek-Nielsen, 1992; Nevard, 1994). These previous studies have also demonstrated associations between head posture and craniofacial morphology. This study's findings suggest that upper cervical spine anomalies may be more common in

Malaysian children with CLP (24%) than in American children (22%) (Horswell, 1991), Scottish children (13%) (Sandham, 1986), and Norwegian children (18%) (Ugar and Semb, 2001). However, it must be stressed that the study groups referred to include different proportions of cleft types so comparisons of incidence should be undertaken with some caution. Furthermore, the present study was based upon 3D CT scans of subjects while earlier studies were based upon 2D cephalometric radiographs. The enhanced clarity offered by CT images may well display anomalies more clearly and thereby facilitate the diagnosis of CLP associated defects. Previous studies have reported similar frequencies of fusion in NC groups or in the general population, ranging from 0.5 – 5% (Gray *et al.*, 1964; Brown *et al.*, 1964; Farman and Escobar, 1982). In contrast, the author did not find any fusion anomalies, probably due to the small sample size of the NC group. However, ethnicity cannot be ruled out as an explanation.

Osborne *et al.* (1971) suggested a smaller than normal anterior arch of the atlas could have a direct effect on the anterior-posterior dimension of the pharynx. The anterior arch of C1 is suggested to play a significant role in the establishment of adequate velo-pharyngeal function and speech in children with CLP (Osborne *et al.*, 1971; Sandham, 1986). These findings suggest that the ossification of anterior arch of C1 may be compromised in patients with CLP and this may later contribute to problems in speech. The importance of the anterior arch of C1 and upper cervical vertebrae was highlighted by Berkowitz (1996) in achieving adequate velopharyngeal closure and speech.

The finding of short vertebral bodies in the cervical spines of infants with clefts is consistent with a delay in growth in infancy. Previous studies have shown a delayed growth in children with clefts of the lip and palate (Bowers *et al.*, 1987; Seth and McWilliams, 1988; Harris and Hullings, 1990; Lilius and Nordstrom, 1992; Neiman and Savage, 1997; Grippaudo and Kennedy, 1999; Spyropoulos and Burdi, 2001).

4.3 Nasopharynx

The findings in relation to the nasopharynx showed that there were increases in the nasopharyngeal space, maxillary tuberosity, the zygoma, and a greater height of the nasopharynx from the posterior part of the vomer (hormion) to hamulus left and right in CLP.

The increased nasopharyngeal space may be associated with compression of nasopharyngeal structures including the eustachian tube. The alteration of the medial pterygoid plate and hamulus may alter the origin and orientation of the tendon

of tensor veli palatini, affecting its function and pull, and lead to eustachian tube dysfunction. These anatomical variations may compromise the dilatory mechanism of the eustachian tube leading to clinical problems such as otitis media and hearing loss. These anatomical variations could also play a possible role in the production of velopharyngeal incompetence, hypernasality and upper respiratory tract infection.

4.4 Cranial Base

Midface hypoplasia is commonly associated with CLP. The cranial base in the CLP group demonstrated smaller heights of the basisphenoid and basioccipital bones and a smaller anterior cranial base distance from sella to nasion which may provide a clue as to the origin of this facial feature. In a normal foetus the cranial base is a border structure between the neurocranium and the facial skeleton. Thus, the development and growth of the cranial base can interact both with the neurocranial and facial skeletal development. The cranial base is derived from the chondrocranium (Sperber, 2001) and the formation of the chondrocranium starts around the 5th foetal week. The elevation of the fusion of the palatal shelves takes place around 7-10 weeks gestational age. At this time no ossification has occurred in the occipital, sphenoid, ethmoid and frontal (Kjaer, 1990, 1992). Kjaer *et al.* (1993) have shown that the human basal cranium undergoes dimensional changes when the palatal processes are elevated, and the primitive face, with its widely-spaced eyes, changes to a face with the eyes closer together.

Since the cranial base develops from the chondrocranium, the possibility cannot be excluded that an inborn alteration or a delayed maturation of the early development of the cartilaginous cranial base affects not only the height of the basisphenoid and basioccipital bones, but also the length of the cranial base, the width of the nasopharynx, and the width of the cranial base and SOS, as all these structures develop from the same basic structure.

The morphologic findings in the cranial base of CLP infants could possibly be ascribed to deficient development of the chondrocranium at the time of cleft formation.

4.5 Spheno-occipital synchondrosis (SOS)

The main difference noted in the spheno-occipital synchondrosis in CLP was a greater inferior width. The SOS is regarded as an important maturity and growth centre of the facial skeleton (Ford, 1958; Stramrud, 1959; Thilander and Ingervall, 1973; Melsen, 1974). Post-natal growth in the SOS is the major contributor to growth in the cranial base, persisting into early adulthood. This prolonged growth period

allows for continued posterior expansion of the maxilla to accommodate future erupting molars and provides space for the growing nasopharynx.

Previous studies have concentrated upon growth and closure of the SOS by examining non-cleft human autopsy specimens (Ford, 1958; Thilander and Ingervall, 1973; Melsen, 1974). The basicranium is also the first region of the skull to reach adult size, and it is the structural foundation of many aspects of craniofacial architecture. As the basicranium grows, it elongates and flexes in the spheno-ethmoid, mid-sphenoid, and spheno-occipital synchondroses (Lieberman *et al.*, 2000).

The greater width found in the inferior part of the spheno-occipital synchondrosis could be related to a defect in the chondrocranium of the cranial base. In the present study, there was a significant difference between males and females in the width of superior SOS. The SOS in females was narrower than in males. Previous studies on autopsy specimens have shown that the SOS starts to fuse, beginning on its cerebral surface, at 12 –13 years of age in girls and 14-15 of age in boys; with ossification of the external aspect complete by around 20 years of age (Thilander and Ingervall, 1973; Melsen, 1974)). It is possible that a delay in skeletal maturation in CLP contribute to its greater width in the inferior region where fusion normally occurs at a later stage. The narrower SOS in CLP females compared with males might then reflect a tendency to earlier skeletal maturation in females.

In this study infants, with CLP tended to have a wider SOS, in contrast to the narrower SOS reported previously in Crouzon syndrome and Apert syndrome Kreiborg *et al.* (1993). A wider SOS could be associated with dysmorphic and compensatory growth changes at a later age.

5.0 Summary of Findings

5.1 Overall Findings

The fact that several craniocervical structures are affected at the same time suggests that clefting may be one aspect of a more general problem. While this study cannot clarify whether the main aetiological factor is genetic or environmental, the reporting of these common features should assist future researchers. This phenotypic study should also assist molecular biologists searching for a molecular basis of CLP by highlighting the fact that several regions of the developing craniofacial complex are affected in CLP.

The phenotypic changes relate to structures derived from the first, second and third arches and may reflect alterations in cartilage growth and/or ossification. The findings of this study suggest there could be a common underlying defect or delay in endochondral ossification. Development of the hyoid bone, cervical spine, nasopharynx, cranial base and SOS all involve endochondral ossification.

This also could explain, in general, the reduced growth potential in CLP. Previous studies have shown a delayed growth in children with clefts of the lip and palate (Bowers *et al.,* 1987; Seth and McWilliams, 1988; Harris and Hullings, 1990; Lilius and Nordstrom, 1992; Neiman and Savage, 1997; Grippaudo and Kennedy, 1999; Spyropoulos and Burdi, 2001).

The principal feature of skeletal and connective tissue in the face is its dual origin from neural crest cells and mesoderm. These cells establish the origins of the skeletal and connective tissues. The cartilages are the first skeletal elements to develop. The induction of cartilage from neural crest cells is often promoted by the product of the epithelium. In the branchial region the morphology of the cartilages is dependent on Homoebox gene activity. Most of these cartilages undergo endochondral ossification (Johnston and Bronsky, 1995).

Two genes, core-binding factor alpha 1 (Cbfa-1) and Indian hedgehog (IHH), have been shown to control osteoblast differentiation. Bone morphogenetic proteins (BMP), members of the transforming growth factor-beta, and fibroblast growth factors (FGR) and their receptors (FGFR) induce bone formation at genetically designated sites (ossification centers) (Sperber, 2001). Delayed onset of osteogenesis will reduce the final size of a bone, and premature onset of osteogenesis will increase its final size. During the 7th week of intra-uterine life, mesenchymal cells condense as a prelude to both intramembranous and endochondral bone formation. Although the

basic shape and size of bones may be genetically determined, extrinsic functional or environmental factors become the predominant determinator of bone form.

Alterations in cartilage growth can lead to a reduced cranial base. Such a defect of the chondrocranium will then have an inhibiting effect on the midface and maxilla producing a dish-faced deformity of the middle third and dental malocclusion (Sperber, 2001).

Overall, the 3D analysis has disclosed new information about phenotypic variation in CLP and shown several significant differences from NC infants. It has also helped to explain the possible reasons for the clinical problems faced by affected children such as aspiration pneumonia, speech problems, otitis media and upper respiratory tract infection.

The question of whether the phenotypic findings in the craniocervical structures are the cause of the CLP, reflect a common underlying aetiological problem, or are an effect cannot be answered definitively. However, the fact that there are several structures affected together suggests that the clefting may be one aspect of a more general problem.

5.2 Isolated Cleft Palate (ICP)

In this study different CLP groups (CL, UCLP, BCLP and ICP) were compared with an NC group. In addition the ICP group was also compared to the other three affected groups. This was based on the fact that ICP seems to have a different aetiology and the defect occurs at a later time during embryogenesis.

The findings for the ICP group compared with the other CLP groups included smaller length of the left greater horn, smaller intervertebral spaces, smaller nasopharyngeal width at various levels, a greater right hamulus angle, a larger vomer-basion distance and a smaller basisphenoid height. These differences indicate that ICP is a related but aetiologically different condition from CLP.

5.3 Comparison between Males and Females

In this study it was found that four variables were larger in the CLP and NC group males than females; lateral pterygoid width, vomer to lateral pterygoid right, interzygomatic distance and superior SOS width. These findings show that, even in the infant stage, there is a tendency for some craniofacial structures in males to be generally larger than females.